IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:	Group Art Unit: 1616
LAZZERI et al.	Confirmation No.: 5744
Serial No.: 10/525,428	Examiner: Brown, Courtney A.
Filed: December 9, 2005	**DECLARATION OF LUCA LAZZERI**
Atty. File No.: 3797CAS-1	(Under 37 CFR § 1.132)
For: "USE OF VEGETABLE FLOUR AS A BIOTOXIC AGENT WITH IMPROVING ACTION"	***Submitted Via EFS-Web***

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I, Luca Lazzeri, declare as follows:

1. I am a co-inventor of the above-referenced U.S. patent application. I am a skilled artisan in the fields of molecular and cellular biology and have contributed to the design, direction, and supervision of the experiments described in section 4 below.

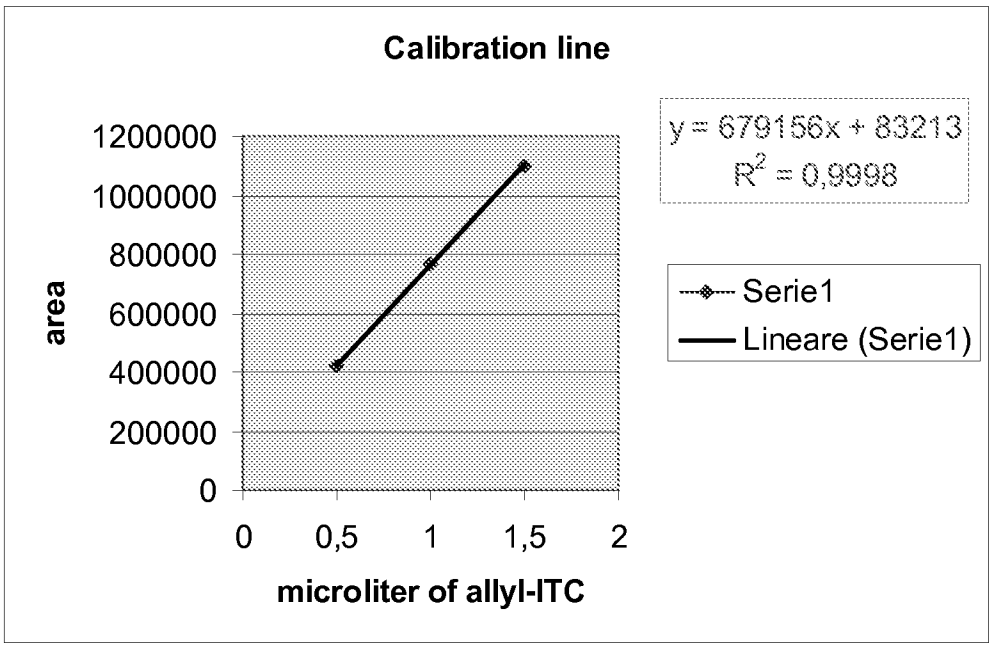
2. This Declaration is being submitted in conjunction with an Amendment and Response to an Office Action having a mailing date of September 4, 2008.

3. The following discussion in sections 4, 5 and 6 are provided in response to the Examiner's rejections of Claims 17-34 and 39 under 35 U.S.C. § 103(a). Specifically, the data presented in sections 4, 5 and 6 demonstrate that my laboratory has produced data showing that the percentage release of isothiocyanate from seed meal that is defatted at a temperature of about 60°C is higher than the isothiocyante released from non-treated seed meal.

4. The trials were performed in an Erlenmeyer flask of one litre closed by a cap with a pierceable septum; 0,2 g of grinded meal were hydrated with 1500 µl of water to activate the hydrolysis reaction. After meal hydration, by a titre syringe gastight for gas injection it was taken 0,5 ml of air in the head space that was injected in Fison mod.HRGC 5300 mega series gas-chromatograph with a FLAME IONISATION detector (FID) using a Rtx 2330 capillary column (30m of length, 0.32 mm Id and 0.2 µm df). The analytical conditions were the following: the flow rate of the carrier gas (He) was 2.0 mL min^{-1}, the injector and detector temperature were 200 and 260 °C respectively. The analyses were carried in isothermal conditions at 70°C, and

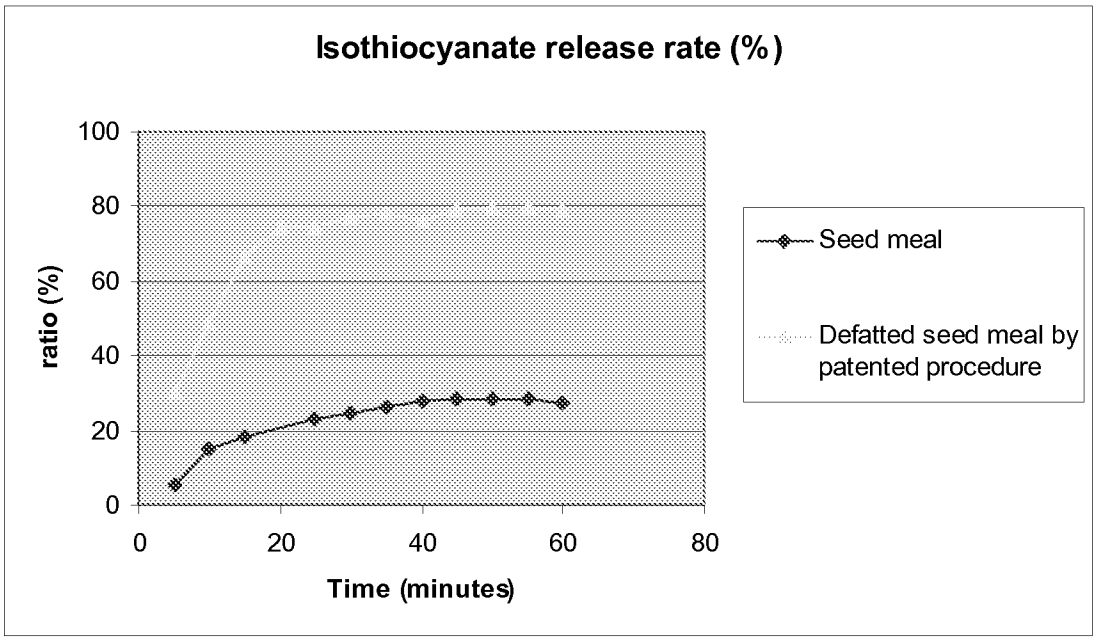
samples were injected in splitless mode. The chromatograms registration and peak calculation were performed by the software Chrom card per Windows version 1.18. Finally, the isothiocyanate amount was calculated by a specific calibration line (Figure 1) previously carried out with different amounts of pure Allyl isothiocyanate (Fluka) operating in the same above reported conditions.

Figure 1



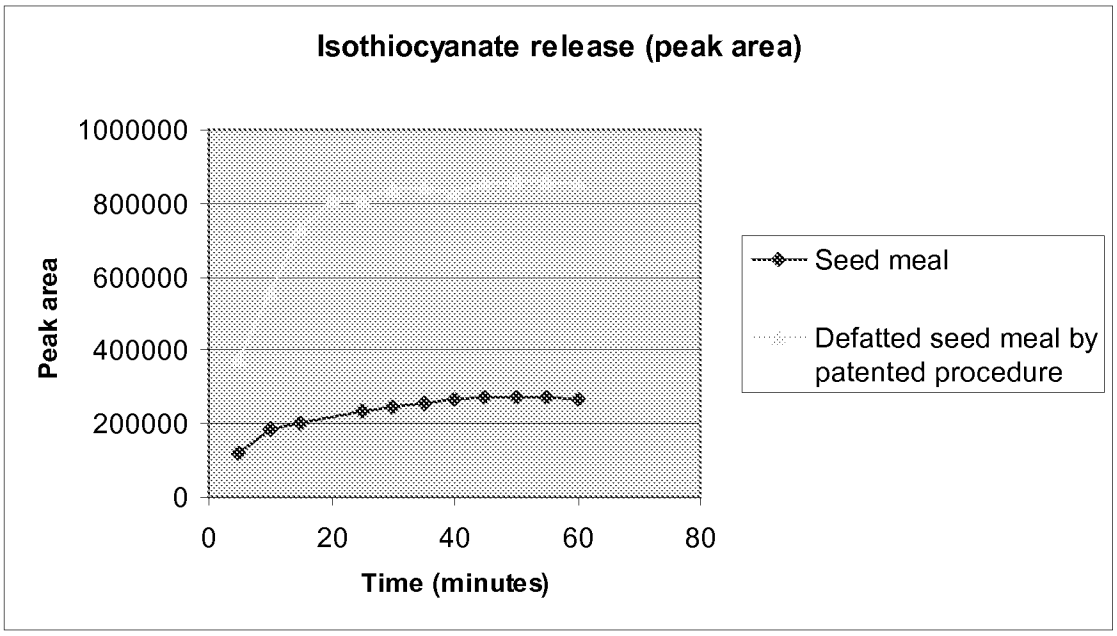
5. Figure 2, below, shows the percentage release of isothiocyanate of non-treated seed meal of *Brassica carinata* A. B. (◆) and a seed meal of *Brassica carinata* A. B. de-oiled at a temperature of about 60°C (▲). The percentage release of isothiocyanate is the percent ratio of the released isothiocyanates and the total content of glucosinolates, which could produce isothiocyanates. It can be easily seen that the seed meal defatted at a temperature of about 60°C shows a percentage release of isothiocyanates much higher than that of un-treated seed meal. It also very important to note that the release of isothiocynates by the seed meal de-oiled at a temperature of about 60°C is very fast. This clearly contributes to the surprisingly high fungitoxic and/or insecticide activity of the seed meal de-oiled at 60°C.

Figure 2



6. Figure 3 shows peak areas of chromatograms at different times of isothiocyanate released by un-treated seed meal of *Brassica carinata* A. B. (◆) and a seed meal of *Brassica carinata* A. B. de-oiled at a temperature of about 60°C (▲). The peak areas are proportional to the quantity of isothiocyanate. Also, the results reported in Figure 3 indicate that the release of isothiocynates by the seed meal de-oiled at a temperature of about 60°C is very fast. The release of isothiocyanate is surprisingly more than three times higher for the seed meal of *Brassica carinata* A. B. de-oiled at a temperature of about 60°C with respect to that of the non-treated seed meal. Please note, that, as a consequence of the of the de-oiling treatment, the seed meal of *Brassica carinata* A. B. loses only the 30% of weight.

Figure 3



7. I hereby declare that all statements made herein of my own are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing therefrom.

Date:_____ By:_____

Luca Lazzeri